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APPLICATION NUMBER: *60/538,687*
FILING DATE: *January 23, 2004*
RELATED PCT APPLICATION NUMBER: *PCT/US05/02317*

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Jon W. Dudas

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Antonio A.	Garcia	Chandler, AZ
Rohit	Rosario	Tempe, AZ
John Devens	Gust, Jr.	Mesa, AZ

Additional inventors are being named on the 1 *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

DUAL RESPONSIVE HYDROGELS WITH STRUCTURAL CHANGES CONTROLLED BY INTERMOLECULAR INTERACTIONS INDUCED BY EXTERNAL FIELDS DURING SYNTHESIS

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ENCLOSED APPLICATION PARTS *(check all that apply)*

[✔] Specification *Number of Pages* 30
[ ] Drawing(s) *Number of Sheets*
[ ] Application Data Sheet. See 37 CFR 1.76
[ ] CD(s), Number
[✔] Other (specify) Return Postcard

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

[✔] Applicant claims small entity status. See 37 CFR 1.27.
[ ] A check or money order is enclosed to cover the filing fees
[✔] The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 17-0055
[ ] Payment by credit card. Form PTO-2038 is attached.

FILING FEE AMOUNT ($): $80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[✔] No.
[ ] Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE Christine M. Meis

Date 01/ 23 / 04

TYPED or PRINTED NAME Christine M. Meis

REGISTRATION NO. *(if appropriate)* 52,024

Docket Number: 112624.00097 PRC

TELEPHONE 602-229-5247

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

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Docket Number	112624.00097 PRO	Type a plus sign (+) inside this box →	+

INVENTOR(S)/APPLICANT(S)		
Given Name (first and middle [if any])	Family or Surname	Residence (City and either State or Foreign Country)
Mark A.	Hayes	Scottsdale, AZ
Manuel	Marquez	Glenview, IL
Zhibing	Hu	Denton, TX

Number 2 of 2

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ASU FULTON school of engineering

Photoresponsive Micro/Nanogels

Antonio A. Garcia (presenter)

Rohit Rosario, Devens Gust, Mark Hayes, Manuel Marquez, Zhibing Hu, Joseph Springer, Tom Picraux, Bruce Bunker (collaborators)

Harrington Department of Bioengineering Seminar
January 23, 2004

ASU Presentation Overview

- Why photoresponsive materials?
- Brief review of prior work
- New phenomena discovered with micro/nano gels
- Current Theory: J-Aggregates
- Musings on Potential Uses
- Conclusions

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ASU Why photoresponsive materials: Some general reasons

- Triggering changes with light – Biology has a 3.6 billion year head start (blue green algae)
  - Structural changes such as state, shape at the nano-to-macro scale
  - Chemical changes for interactions with water and solutes
- Information on environmental change – Remote sensing

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ASU Why you can see this presentation

- Rhodopsin generates an optic nerve impulse in visual receptors of Mollusks, arthropods, and vertebrates
- In rhodopsin, the retinal chromophore is embedded in a pocket formed by 7 helices, which contain about 305 amino acids

Images from Rachel Cassidy and Regina Frey, Department of Chemistry, Washington University, St. Louis, MO 63130

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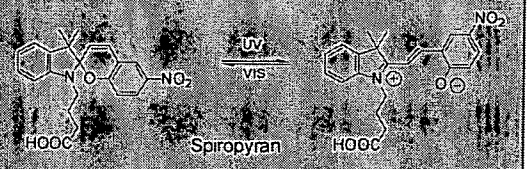
ASU Prior Work: Spiropyran Photochemistry



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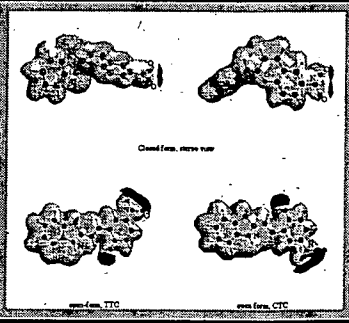
ASU Prior Work: Calculation of Dipole Moment



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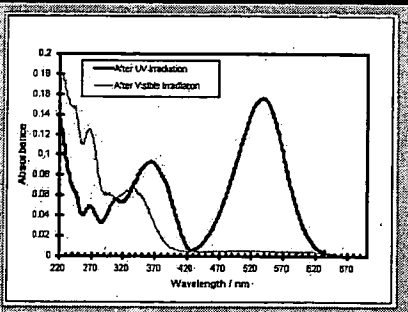
Prior Work: Spiropyran in Ethanol



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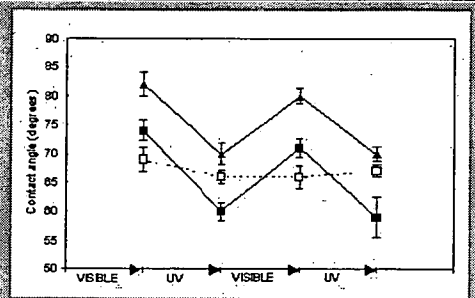
Prior Work: Attaching Spiropyran to Surfaces



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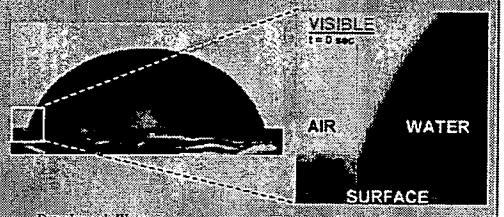
Prior Work: Real-time Water Drop Advance



Drop Liquid: Water
Substrate: Glass
Substrate treated with: TBDS + ADES + SPIRO

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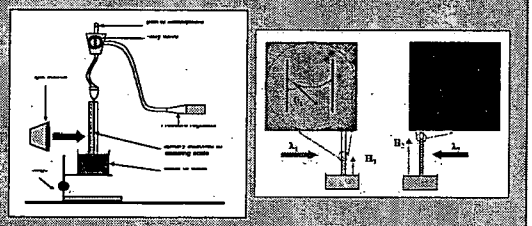
Prior Work: Photocapillarity



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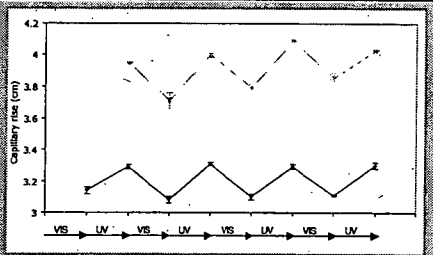
Prior Work: Photocapillary Rise



Effect of repeated light cycling on capillary rise changes

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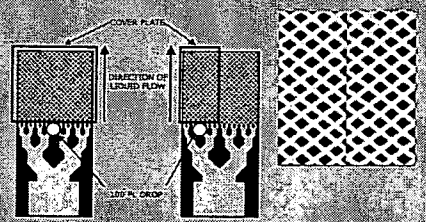
Prior Work: Capillary Networks



a) Cover plate covering the full network
b) Cover plate covering half the network

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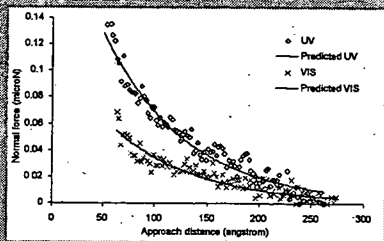
Prior Work: Interfacial Force Microscopy



$$F(D)=\frac{AR}{6D^2}+\frac{2\pi R}{\varepsilon\varepsilon_0 K_D}[(\sigma_1^2+\sigma_2^2)\exp(-2K_D D)+2\sigma_1\sigma_2\exp(-K_D D)]-RK\exp(-\frac{D}{\lambda})$$

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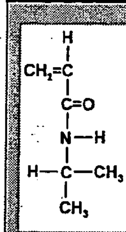
PNIPAM Hydrogels



- From Pelton's review, 2000
  - "In 1978 Philip Chibante, a high school summer student, with aspirations to become a dentist, prepared the first reported temperature-sensitive aqueous microgel under the author's supervision. The microgel was a monodisperse colloidal dispersion based on cross-linked poly N-isopropylacrylamide, herein called polyNIPAM, a polymer which has a lower critical solution temperature LCST in water of 32C. All the microgel properties were sensitive functions of temperature over the range 15-50 C.
- Collaboration with Zhibing Hu and Manuel Marquez led to attaching Spiropyrans to N-Isopropylacrylamide (PNIPAM) hydrogels
  - Large slabs of gel - Macrogel
  - Micro/Nanogel particles
- These gels are thermosensitive
  - Change size with temperature from 15 - 35 C
- Other responsiveness is also possible
  - Light
  - Electric Fields

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Summary of Micro/Nanogel Synthesis

- Micro/Nanogel Synthesis Under Visible Light
  - Upon Irradiation with UV, particle swells
  - Particle becomes charged
  - Easily explained due to osmotic pressure buildup
- Micro/Nanogel Synthesis Under UV or Dark
  - Upon irradiation with UV, particle shrinks
  - Particle becomes charged
  - New phenomena observed, defies conventional wisdom

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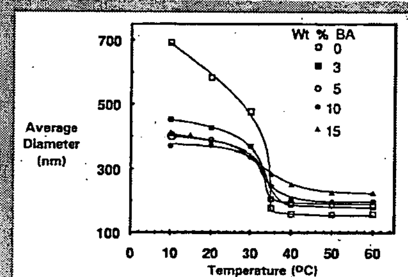
From Pelton, *Adv. Colloid Inter. Sci.*, 85 2000, 1-33



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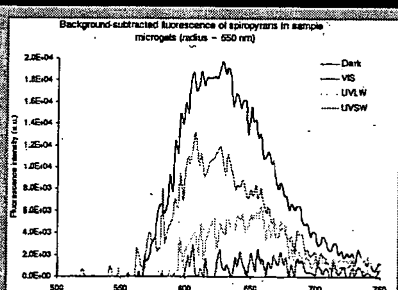
Photochromism within Hydrogel



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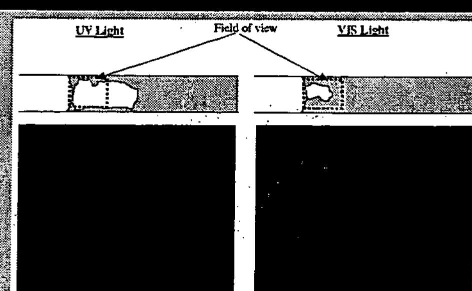
Macrogel behavior with UV and VIS Light



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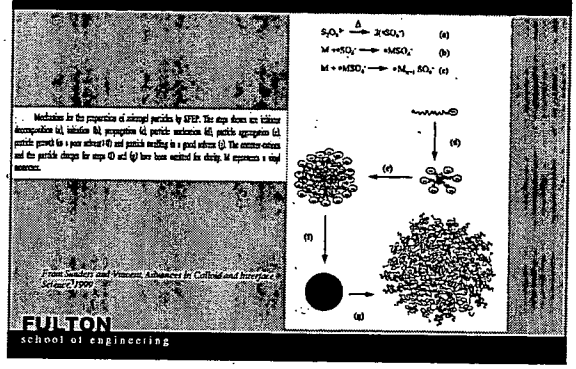
Production of NIPAM Microgels



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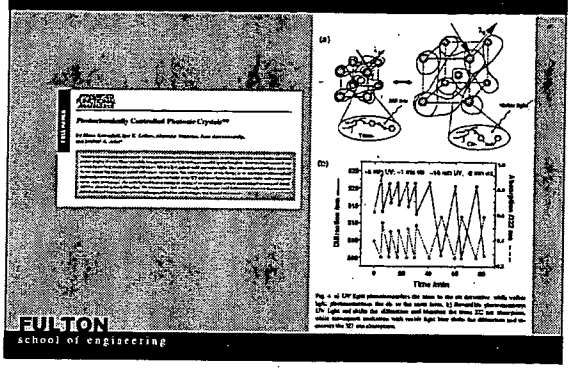
Other Photoactive Microgel Work



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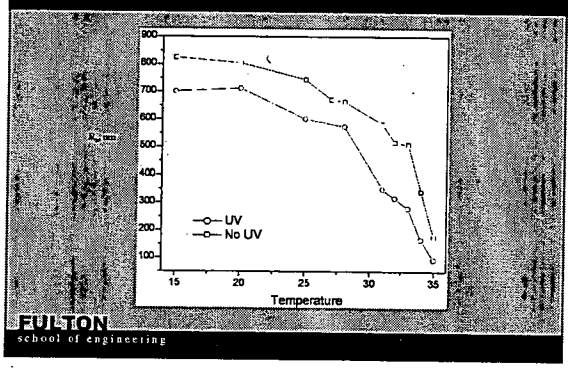
Startling results with Micro/nanogels: Shrink with UV Irradiation



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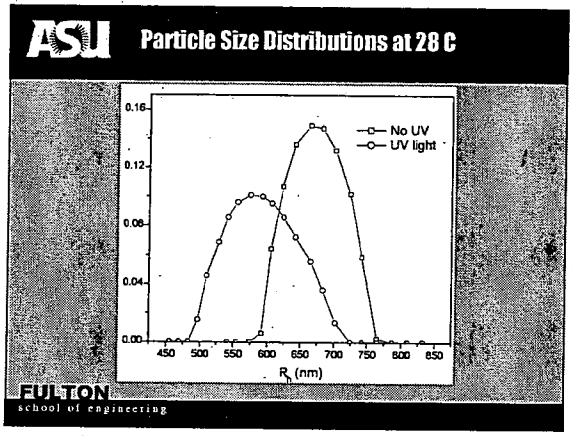
Particle Size Distributions at 28 C



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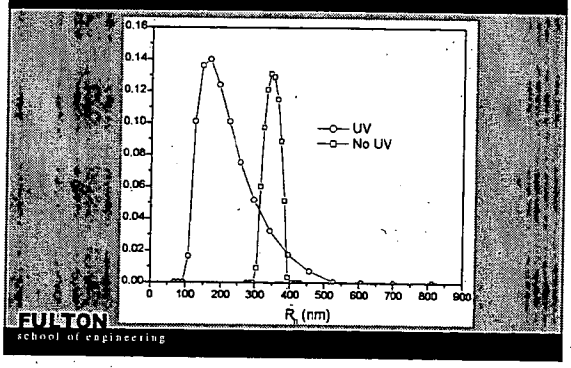
Particle Size Distributions at 34 C



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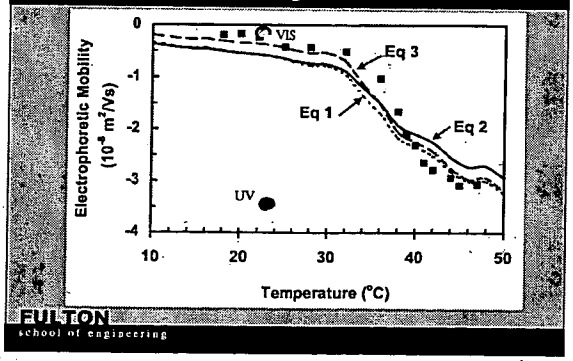
Pelton and Our data : Change in Eletectrophoretic Mobility with Temperature/Light



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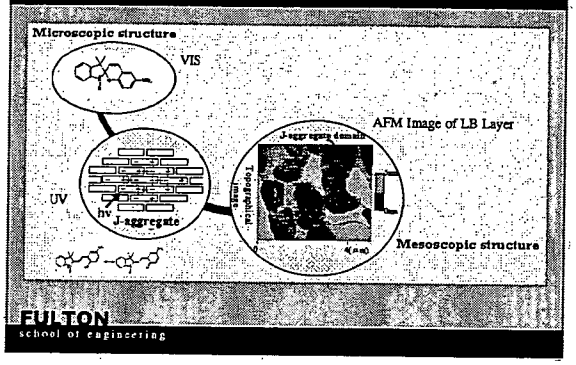
J-Aggregates in Langmuir-Blodgett Films



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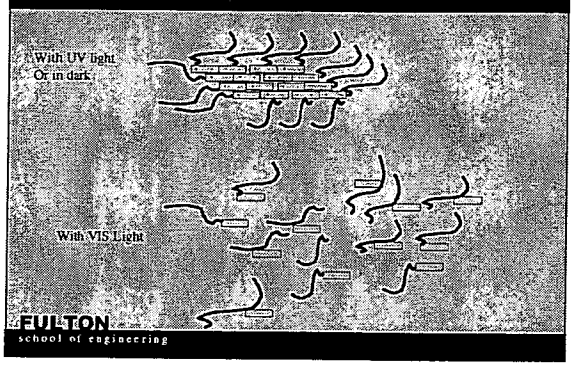
J-Aggregate Theory: Template Formed Upon Synthesis



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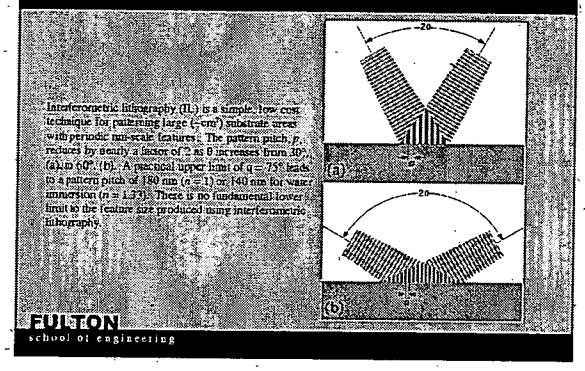
Interference Lithography



Interferometric lithography (IL) is a simple, low cost technique for patterning large (~cm²) substrate areas with periodic nm-scale features. The pattern pitch, p, reduces by nearly a factor of 2 as θ increases from 30° (a) to 60° (b). A practical upper limit of $q = 75°$ leads to a pattern pitch of 180 nm ($n = 1$) or 140 nm for water immersion ($n = 1.33$). There is no fundamental lower limit to the feature size produced using interferometric lithography.

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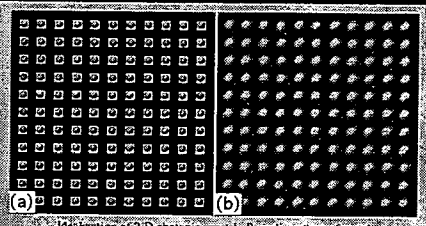
Photonic Crystal



Idealization of 2-D photonic crystal. Periodic pattern of metal pads (white squares) is produced by interference lithography. Electrostatic or chemical attachment of photothermoresponsive hydrogel spheres to the metal pads: shown after shrinking via UV light irradiation in (a). (b) Growth of nanogel spheres after VIS light irradiation.

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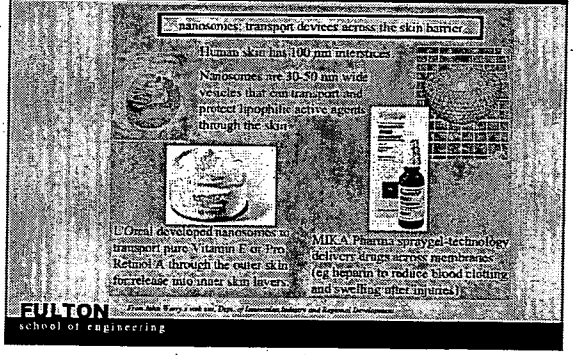
Possible Use: Drug Delivery



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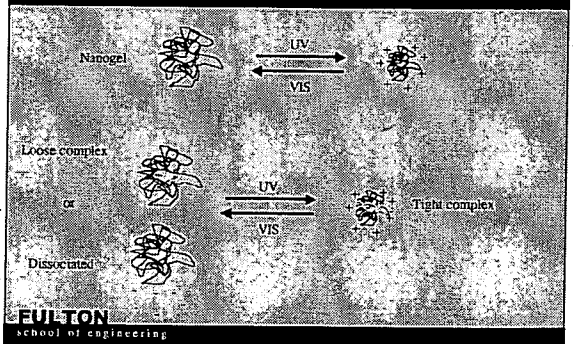
Gene Delivery



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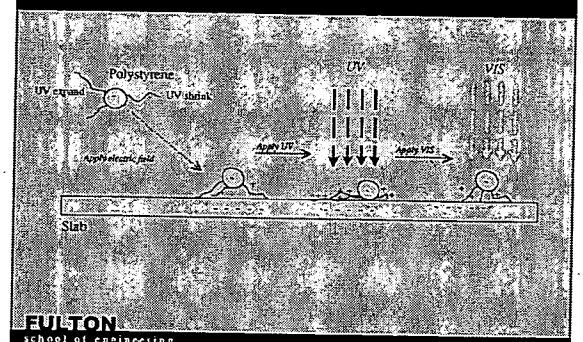
Brownian Ratchet-Based Crawler



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Conclusions

- Micro/Nanogels that shrink or grow with UV light can be synthesized.
- More work will be needed to determine whether the J-aggregate theory suffices to explain shrinkage with UV light.
- Many possible uses can imagined based on photo + thermo responsiveness and with inducible net charges at physiologic pHs.

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Acknowledgements

- Devens Gust, Mark Hayes - Department of Chemistry and Biochemistry
- Manuel Marquez – Los Alamos National Laboratory, Harvard U., Kraft Foods Inc.
- Zhibing Hu - University of North Texas
- Tom Picraux - Chemical and Materials Engineering
- Rohit Rosario - Harrington Department of Bioengineering
- Joseph Springer – Glendale Community College
- Bruce Bunker – Sandia National Laboratory

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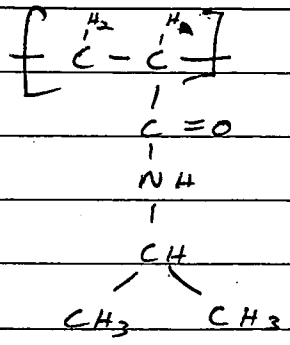
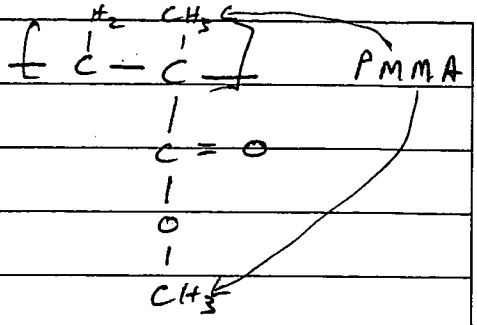
Questions & Comments

N, Isopropyl acrylamide

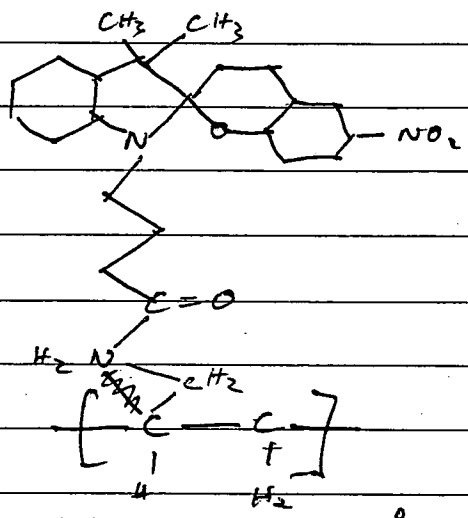
NIPA

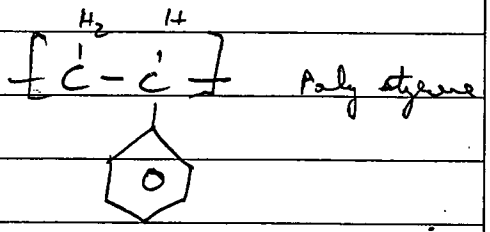




SP allylamide

SP - allylamide

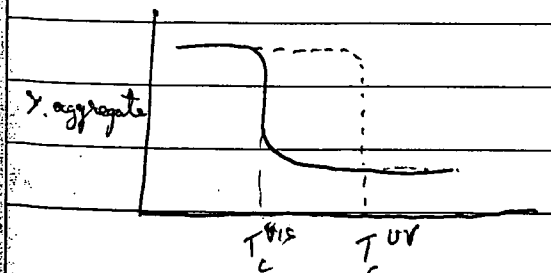




The LCST of NIPA is ~ 31°C (T_c)

It is the temperature at which the hydrogen bonding between the polymer chain and water equals the hydrophobic bonding between the polymer chains.

It is our <u>hypothesis</u> that the LCST of NIPA gels containing SP-allyl pendant groups may be modified by wavelength of light due to dipole / charge creation removal.



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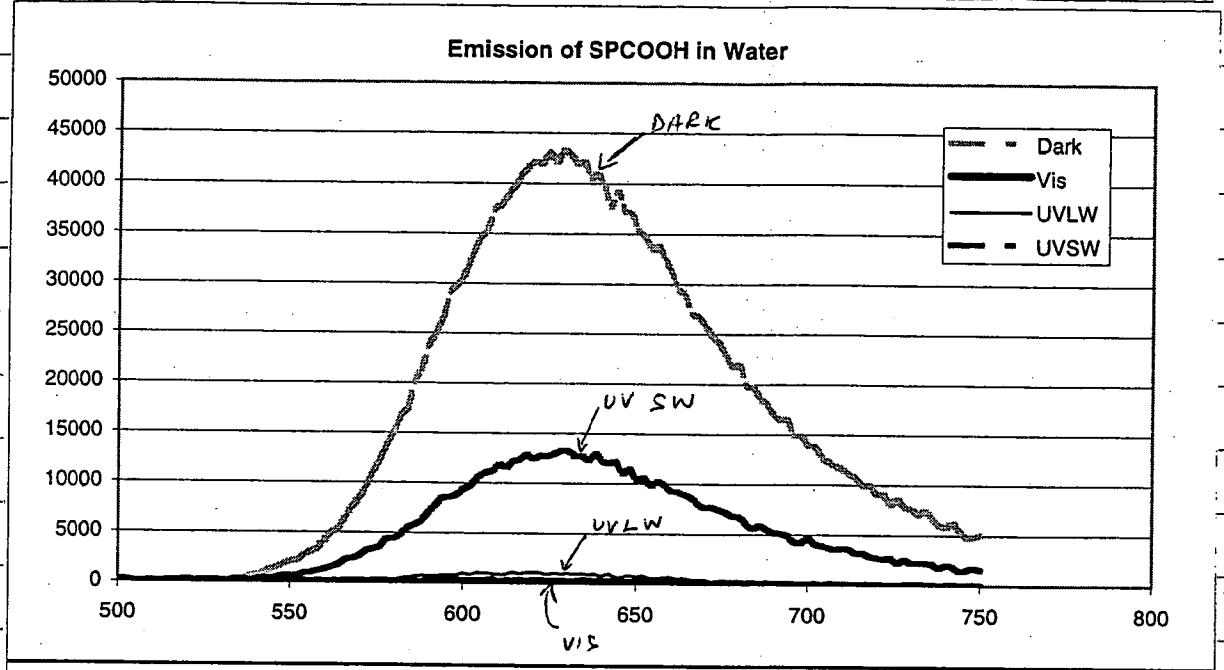
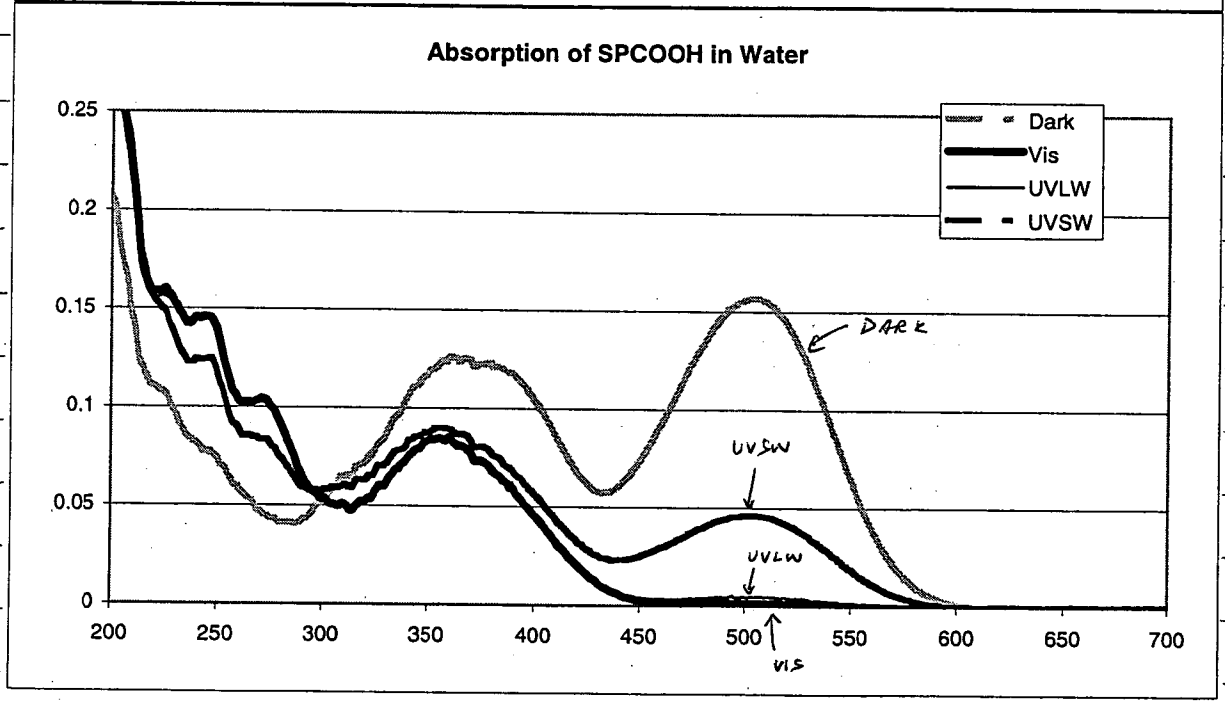
Read and Understood By

Signed	01/13/04	Date	Signed		Date
			Zahit	8/25/03	

Our measurements of SP-COOH in water -





Comparing the spectra, we may need to

① Use VIS & UV light to switch the NIPA-SA gel

② Use fluorescence to detect switching.

This will tell us if the spiro in the gel is active

Continued on Page

Read and Understood By

Witnessed			
04/13/04		Dalit	8/26/03
Signed	Date	Signed	Date

Samples Received from Prof. Zhibing Hu

Sample 1 : 5% NIPA gel w/ 5 mg SA-allylamide

Sample 2 : 5% NIPA gel w/ 8 mg SP-allylamide

Sample 3 : 5% NIPA gel w/ 22 mg SP-allylamide

Sample 4 : 5% NIPA gel w/ unknown SP-allylamide

- The samples looked transparent & pale yellow in color with undissolved clumps of spiropyran (particles) which were deep red in color.
- When the sample tubes were heated by holding them at a particular temperature they suddenly clouded up and became opaque & white. This could be reversed by cooling. The time scale for this transformation was of the order of 10 - 25 sec.
- The samples were stored in tightly sealed tubes at 5°C and equilibrated to room temperature before any testing.

Continued on Page

Read and Understood By

Witnessed 01/13/04
Signed Date

Rohit 9/5/03
Signed Date

Aim - To examine whether the NIPA-SP gels showed changes in their emission under UV and VIS irradiation.

ROOM LIGHT

Sample examined: Sample 1, 5% NIPA gel w/5 mg SP-allylamine

Settings: 20X objective

Bright field & fluorescein

1 second read fluorescence.

N2.1 filter cube

590-665 nm emission

515-560 nm excitation

Method:

VISIBLE LIGHT 15 MIN. 515-560nm

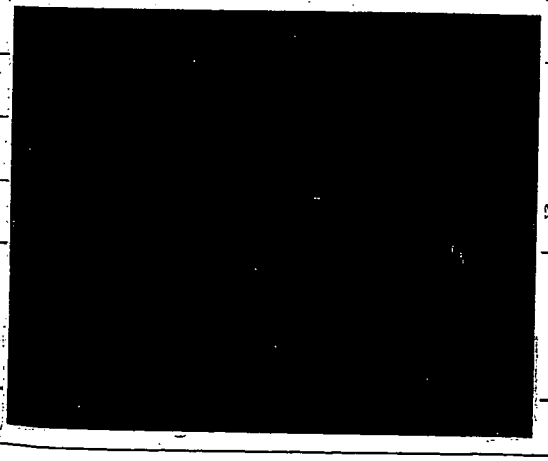
A small piece of gel was smeared onto a microscope slide, a drop of water was placed over it and it was irradiated with the chosen wavelength.

UV LIGHT 15 min. N366 nm

UV source was a hand-held lamp in longwave setting

Vis source was high pressure mercury lamp with 515-560 nm filters.

The UV lamp was turned off during the 1s. emission reading.



VISIBLE LIGHT 15 MIN. 515-560nm

Continued on Page 6

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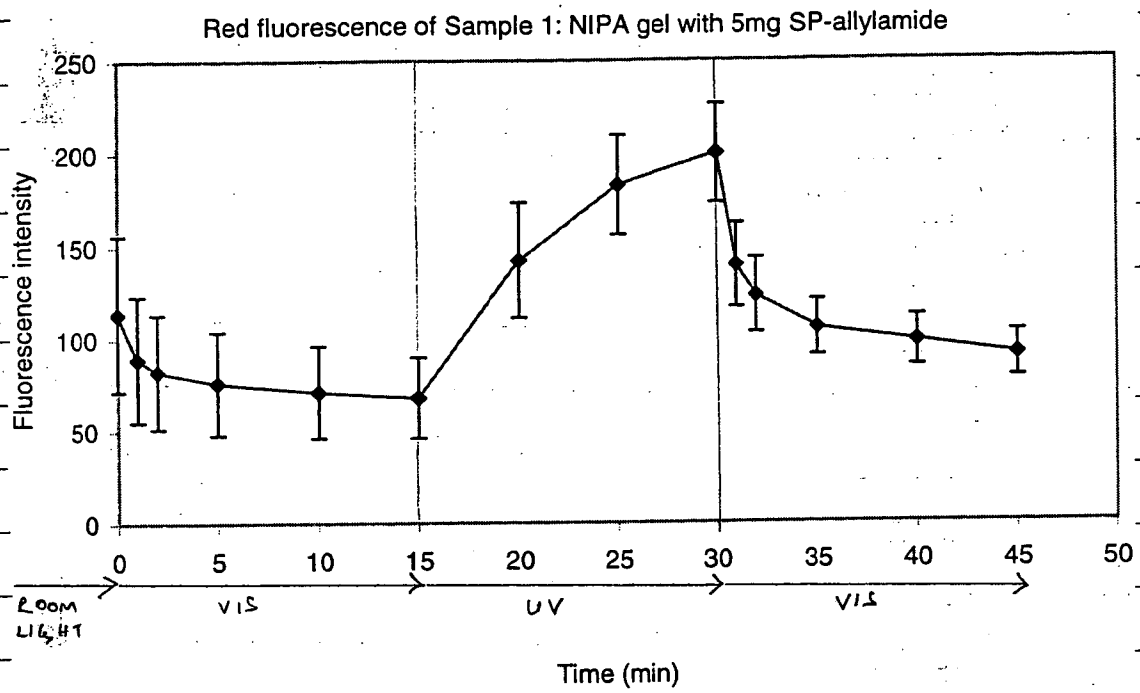
Notebook No. 1

Continued From Page 5



Light condition	Time (min)	Average	Std Dev.
Room Light	0	113.96	42.20
Vis 1min	1	89.22	34.23
Vis 2 min	2	82.43	31.10
Vis 5 min	5	75.98	28.04
Vis 10 min	10	71.05	24.89
Vis 15 min	15	68.07	21.85
UV 5 min	20	142.42	31.17
UV 10 min	25	183.00	26.78
UV 15 min	30	199.76	26.42
Vis 1 min	31	139.51	22.70
Vis 2 min	32	123.15	20.17
Vis 5 min	35	105.63	15.08
Vis 10 min	40	98.50	13.60
Vis 15 min	45	91.09	12.38

The spectrum switches between open (fluorescent) and closed (non-fluorescent) forms with light.

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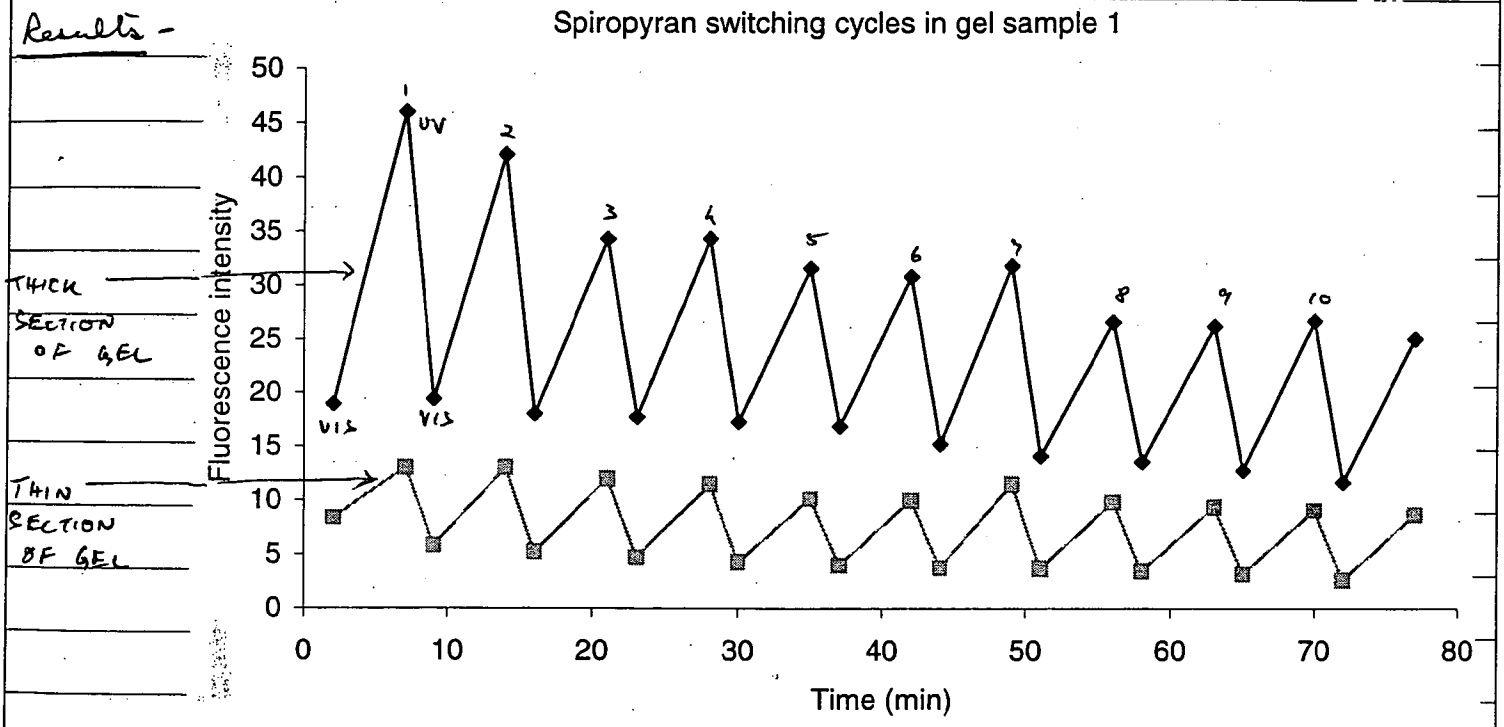
Aim - To study how many times the NIPA-SA gel could be switched back & forth with UV and vis light.

Sample used : Sample 1

Method : The gel was smeared onto a glass slide, covered with water and irradiated with either UV (5 min) or vis (2 min) in between readings. 20x objective & 1 sec. exposure used. A thick section and a thin section of the gel were examined using epifluorescence microscopy.

Results -



The spiropyran can be switched several times back & forth (at least 10). There is some degradation in its ability to open - may an effect of prolonged exposure to intense light.

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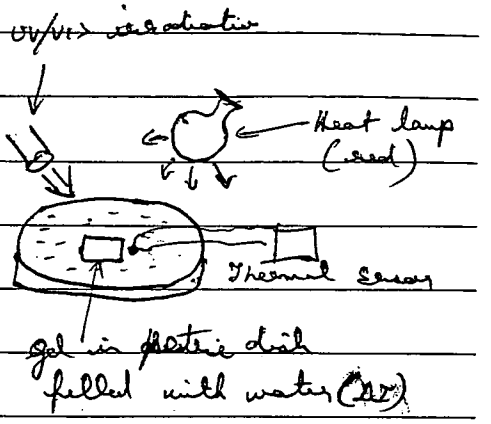
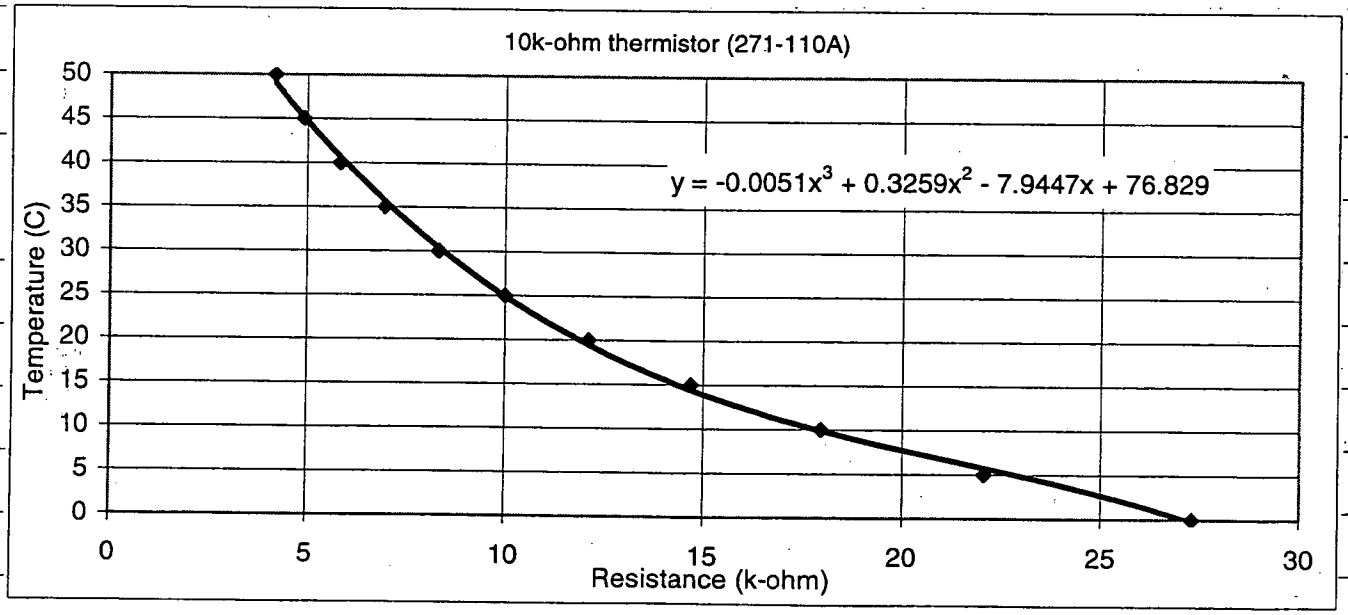
Aim - To find if the LCST of Sample 1 gel is affected by the wavelength of incident light.

Method - The experimental set-up is shown in the sketch. The % aggregation in the polymer gel was estimated visually as the temperature was either slowly increased (heat lamp on) or cooled (heat lamp off). Temperature was measured using a 10 k-ohm thermistor (271-110A) which was placed near the gel in the beaker, and the resistance read off a voltmeter. Equilibration was for 10 min before taking readings.



Calibration curve for thermistor -



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Results –

VIS % aggregation	k-ohms Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
80	7.66	7.76	7.49	7.6	7.53
60	7.8	7.83	7.62	7.64	7.6
40	7.93	7.89	7.72	7.69	7.75
20	8.01	8.04	7.86	7.79	7.84

VIS % aggregation	Temperature deg. C Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
80	32.80276	32.41987	33.46325	33.03449	33.30675
60	32.26788	32.15432	32.95708	32.87983	33.03449
40	31.77846	31.92831	32.57253	32.68745	32.45797
20	31.48073	31.36975	32.04113	32.30582	32.11655

VIS % aggregation	VIS	S.D.
80	33.00542	0.413882
60	32.65872	0.41421
40	32.28495	0.405698
20	31.86279	0.412777

UV % aggregation	k-ohms Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
80	7.15	7.27	7.2	7.36	7.35
60	7.36	7.34	7.28	7.42	7.43
40	7.52	7.37	7.36	7.53	7.48
20	7.55	7.42	7.45	7.63	7.58

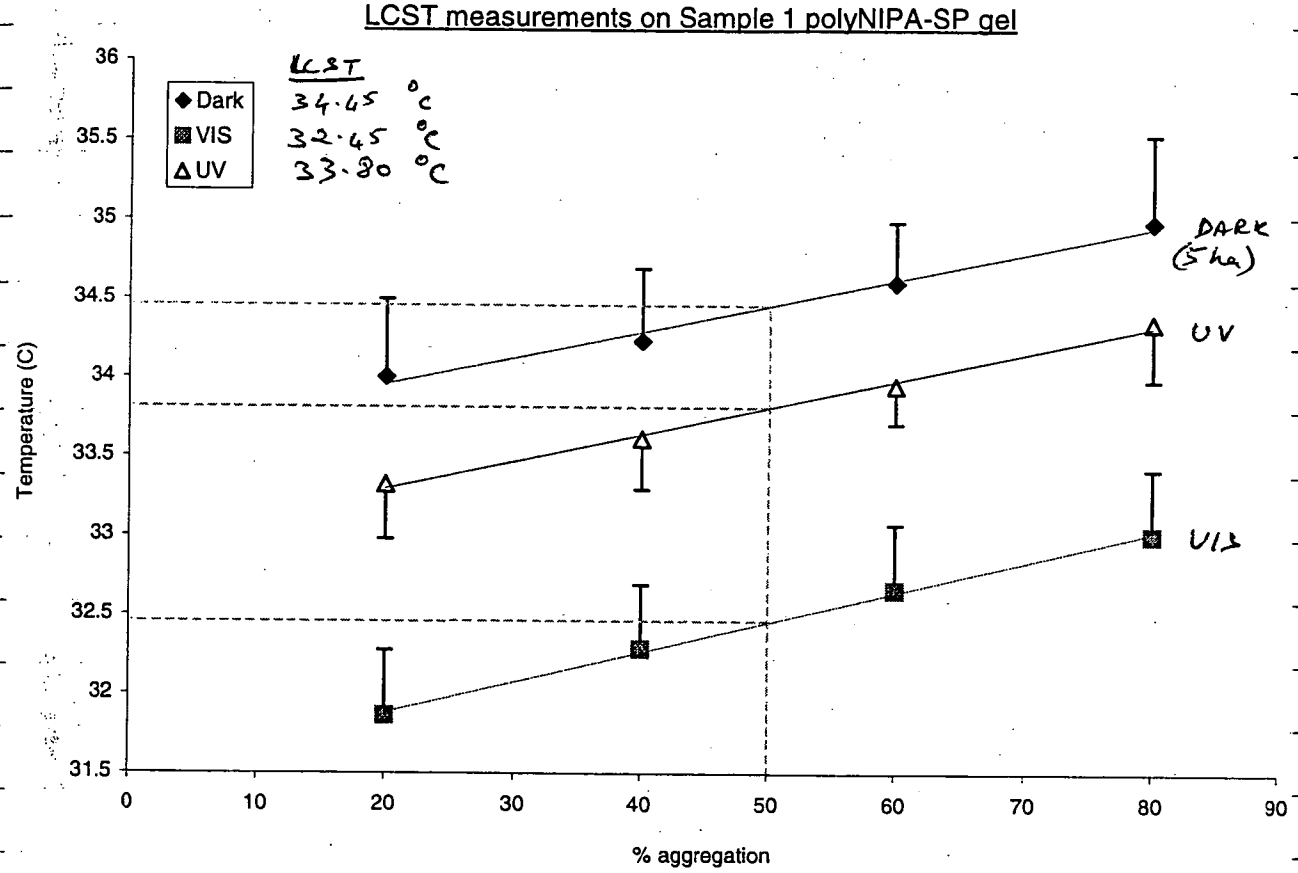
UV % aggregation	Temperature deg. C Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
80	34.82104	34.33616	34.61825	33.97657	34.01635
60	33.97657	34.05618	34.29604	33.73876	33.69928
40	33.34581	33.93683	33.97657	33.30675	33.50248
20	33.22875	33.73876	33.62043	32.91843	33.11207

UV % aggregation	UV	S.D.
80	34.35368	0.369028
60	33.95337	0.244515
40	33.61369	0.321886
20	33.32369	0.345823

Dark % aggregation	k-ohms Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
80	7.1	7.29	6.93	7.06	7.17
60	7.22	7.35	7.09	7.17	7.19
40	7.28	7.48	7.17	7.31	7.24
20	7.33	7.53	7.21	7.42	7.28

Dark % aggregation	Temperature deg. C Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
80	35.0249	34.25595	35.7262	35.18878	34.73979
60	34.53744	34.01635	35.06581	34.73979	34.65872
40	34.29604	33.50248	34.73979	34.17592	34.4568
20	34.09605	33.30675	34.57782	33.73876	34.29604

Dark % aggregation	~~UV~~ DARK	S.D.
80	34.98713	0.544083
60	34.60362	0.382249
40	34.23421	0.460381
20	34.00308	0.494859



If the gel was held at ~ 33°C it should be possible to switch it between aggregated and non-aggregated states.

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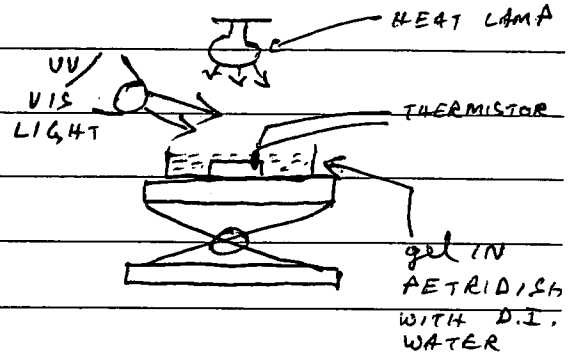
Aim - To switch the poly NIPA-SP gel (sample 1) between aggregated and non-aggregated states using light while holding it at a temperature near its LCST.

Method - A slab of gel was soaked in D.I. water overnight in the dark. It appeared that most of the SP particles were removed from the gel and were suspended in the water. The gel was fully hydrated, pale yellow and transparent. The distance between the gel and the heat lamp was used to precisely control the temperature. Depending on the wavelength of irradiation, the distance of the gel from the heat lamp was adjusted to maintain the temperature.



The % aggregation was estimated visually by approximating the fraction of the gel that had turned from transparent to white.

The temperature was held at ~~33.2~~ 33.21 °C (S.D. = 0.1 °C) and the wavelength of light cycled between UV and VIS. This resulted in the gel aggregating (under VIS) and getting rehydrated (under UV).

This is a demonstration of control of the LCST using light.

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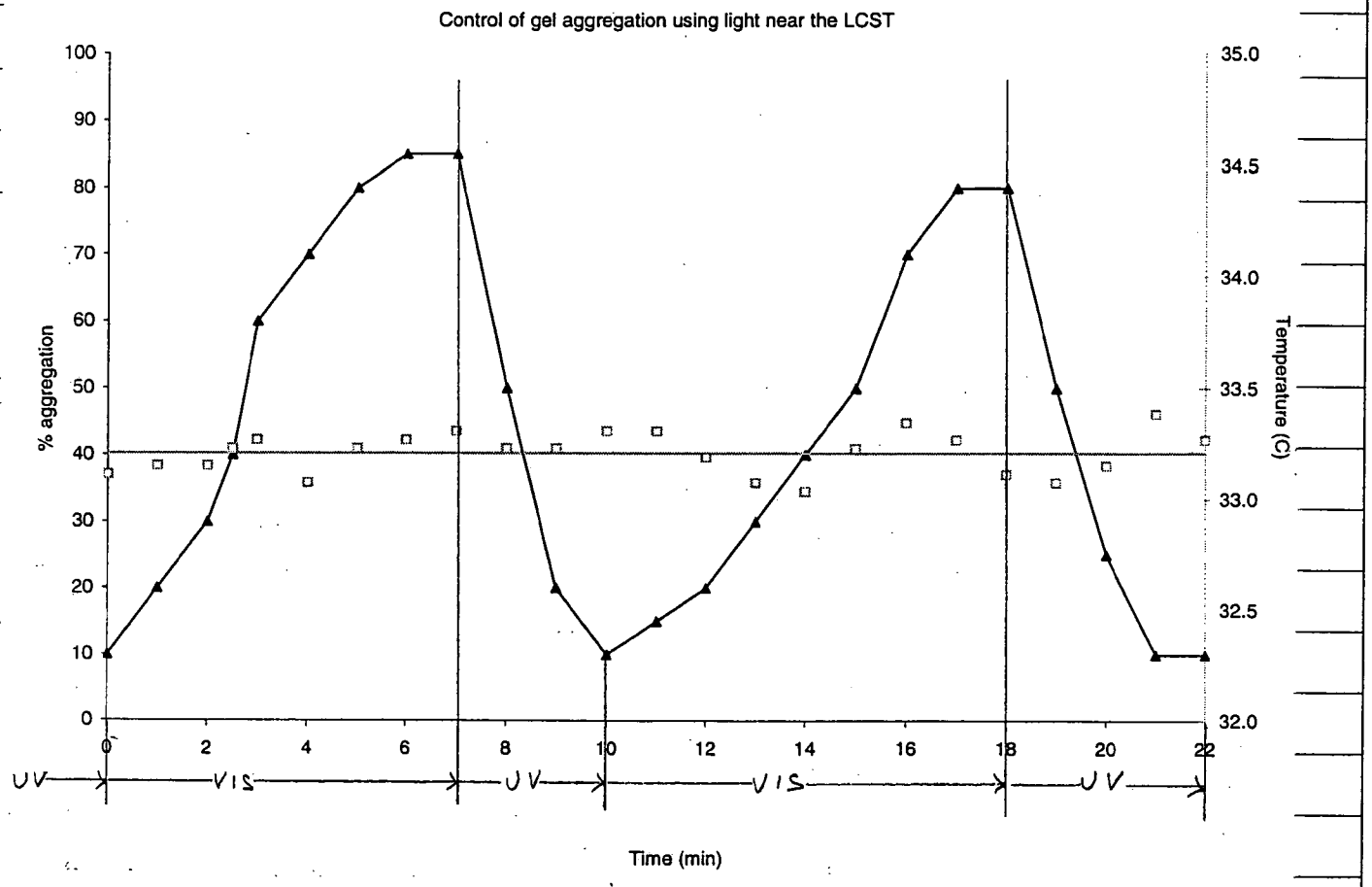
Results –

Light	Time (min)	Resistance (k-ohm)	Temperature (deg C	% aggregated	Average Temperature (deg C)
UV	0	7.58	33.11	10	33.21
VIS	1	7.57	33.15	20	33.21
	2	7.57	33.15	30	33.21
	2.5	7.55	33.23	40	33.21
	3	7.54	33.27	60	33.21
	4	7.59	33.07	70	33.21
	5	7.55	33.23	80	33.21
	6	7.54	33.27	85	33.21
	7	7.53	33.31	85	33.21
UV	8	7.55	33.23	50	33.21
	9	7.55	33.23	20	33.21
	10	7.53	33.31	10	33.21
VIS	11	7.53	33.31	15	33.21
	12	7.56	33.19	20	33.21
	13	7.59	33.07	30	33.21
	14	7.60	33.03	40	33.21
	15	7.55	33.23	50	33.21
	16	7.52	33.35	70	33.21
	17	7.54	33.27	80	33.21
	18	7.58	33.11	80	33.21
UV	19	7.59	33.07	50	33.21
	20	7.57	33.15	25	33.21
	21	7.51	33.38	10	33.21
	22	7.54	33.27	10	33.21



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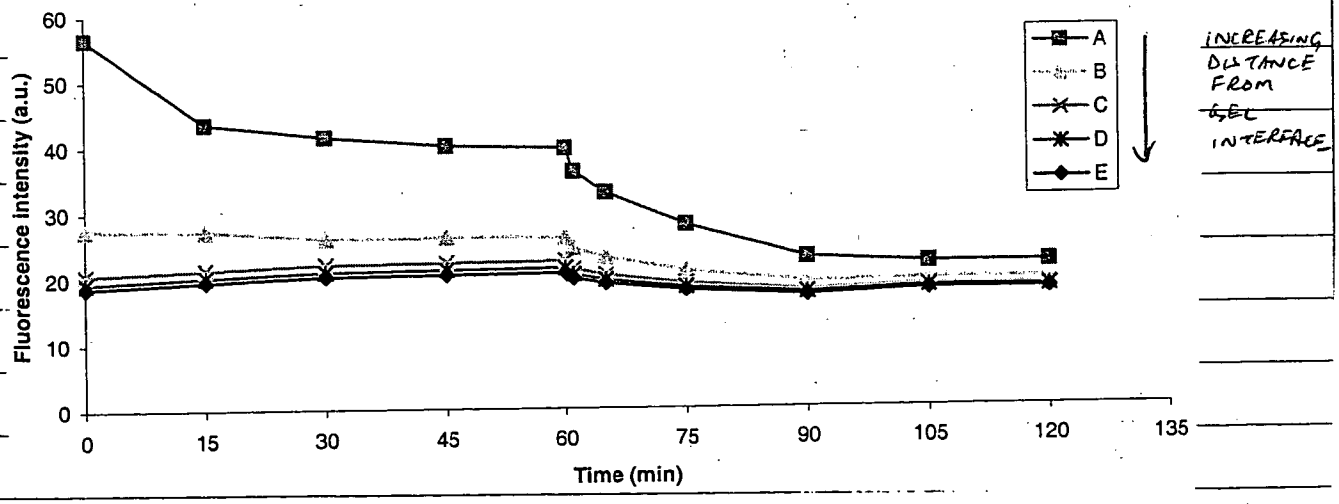
Aim - To examine the retention of GFP in SF-gels under UV & VIS

Method - GFP solutions were made by adding 50 µl GFP (4 mg/ml) to 1 ml HEPES buffer to make a final concentration of 46 µM. Enhanced GFP has excitation peaks at 280, 400, 489 nm (brightest) and emission at 517 nm. Molecular weight of GFP = 31,000 g/mol and ε_{280nm} = 21050.

Pieces of gel were soaked in the dark in the GFP solution for 30 min. A piece of the gel was then soaked into a 25 µl micropipette tube and DI water was sucked into the tube after it. The gel/water interface was then examined using a 5x objective, FI/RH filter cube with a 5 sec integration time, and with a N2.1 red filter while they were irradiated with either UV or VIS for 1 hour each.

Results -



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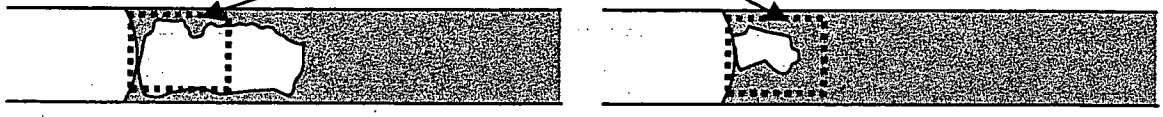
Green and Red fluorescence images of gel under UV and VISIBLE irradiation.

14

Fluorescence images of PolyNIPA-spiro gel in ~1.2 mm dia. tube filled with water. The gel had been soaked in 6 μM green fluorescent protein (GFP) solution for 30 min. At ~33°C, the gel could be switched between aggregated form (VIS light) and hydrated form (UV light) for 2 cycles. After that the photoswitching stopped, and the spiropyran could not be "closed" with VIS light. Possibly, the GFP is reacting with the open form of spiropyran over time and preventing it from being closed with light.

UV Light Field of view **VIS Light**



GREEN FL.

OVERALL	74.66 ± 7.69
LEFT	74.64 ± 4.39
RIGHT	76.73 ± 3.99

GREEN FL.

OVERALL	74.96 ± 22.21
LEFT	133.19 ± 13.51
RIGHT	65.95 ± 3.14

1 Page

01/13/04
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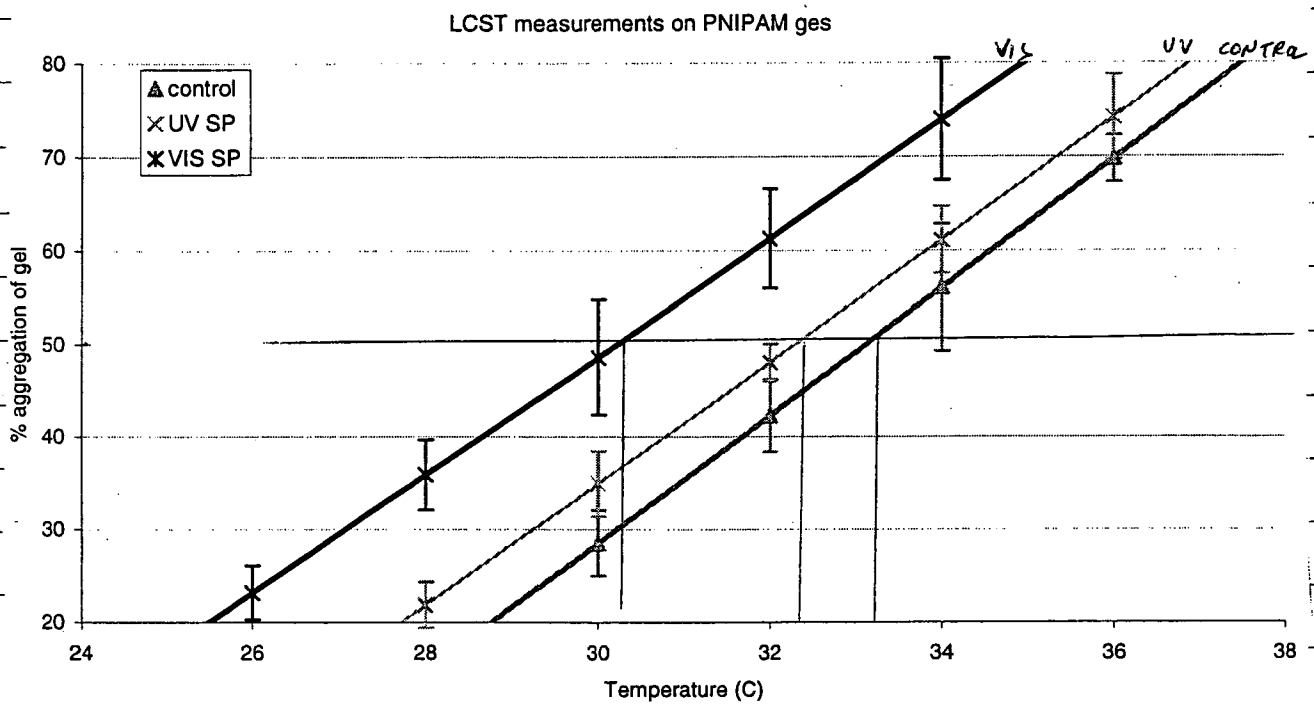
Aim - To examine the effect of temperature on LCST of new samples of SP-gels.

Method - The new samples of the SP-gel did not have particles of undissolved speciapyran. They were yellow compared to the white / transparent control gels. A control gel was scanned simultaneously to the SP-gel. Method same as on page 10.

Results -



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Samples Received from Prof. Zhibing Hu along with light scattering result.

Sample 1. (10-14-03)

0.6 g NIPA, 0.006 g spiropyran and BIS 0.013g into 25 ml DI water. The reaction was taken at temperature at 70 °C under N_2 gas for 4 hours. Particle size(~650 nm at 23 °C)

Sample 2.

0.4 g NIPA, 0.006 g spiropyran and BIS 0.013g into 25 ml DI water. The reaction was taken at temperature at 70 °C under N_2 gas for 4 hours. (~ 550 nm 23 °C)

Sample 3.

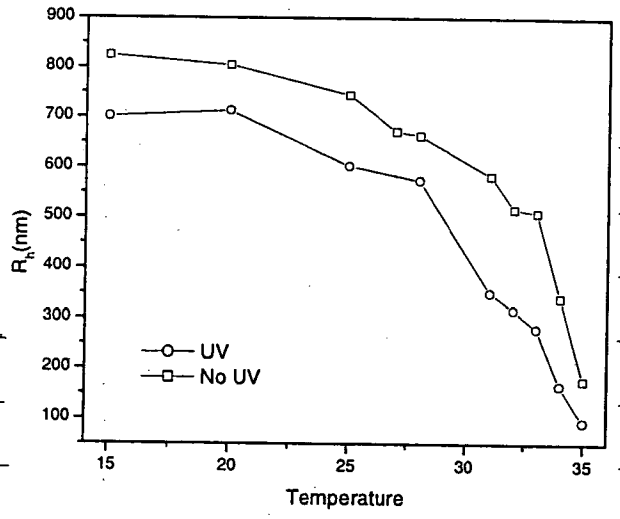
0.4 g NIPA, 0.006 g spiropyran and BIS 0.013g into 25 ml DI water. The reaction was taken at temperature at 70 °C under N_2 gas for 4 hours. (This reaction was under UV light) (~ 470 nm 23 °C)

Sample 4.

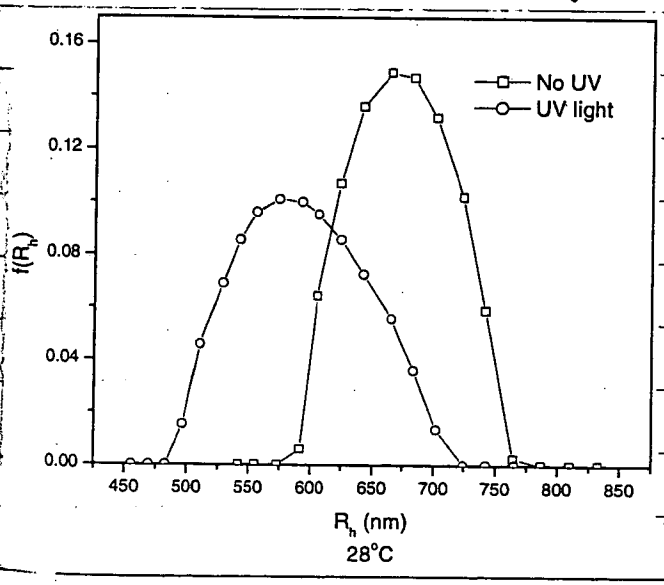
5% NIPA and 0.3% Bis with 1.35 mg spiropyran gel.

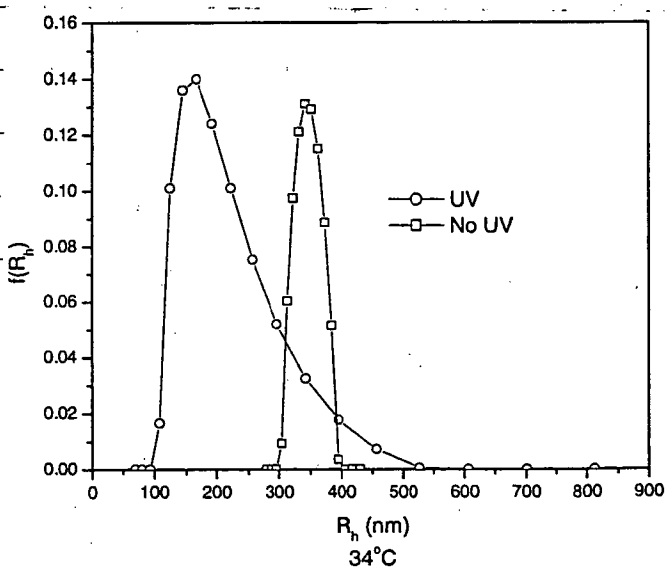
Sample 5.

5% NIPA with 0.3 % Bis Gel.



Light scattering results: The microgels shrink under UV while macrogels





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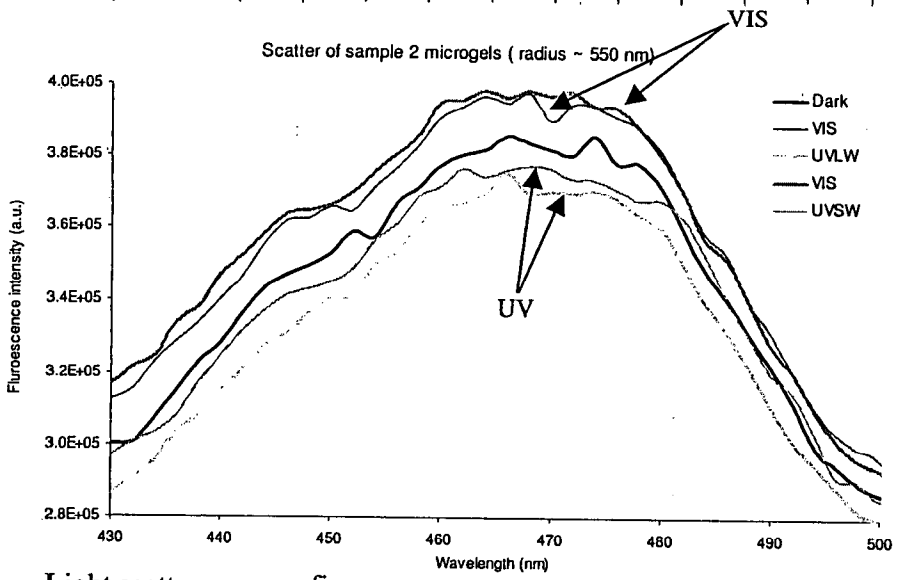
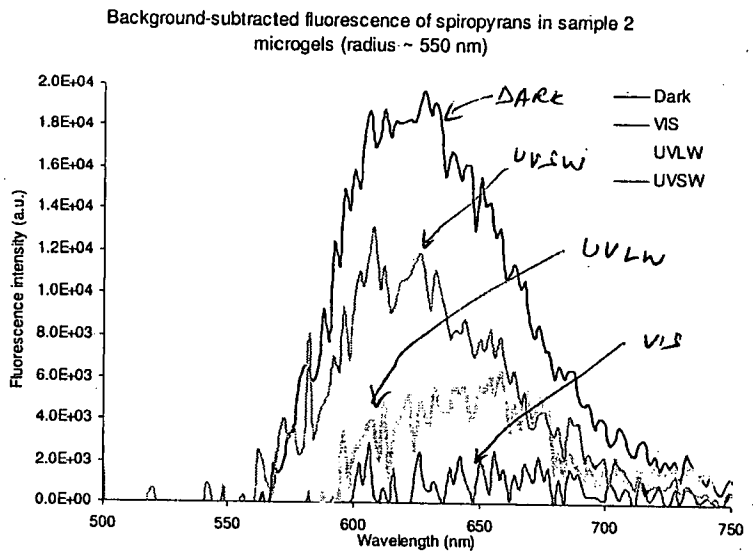
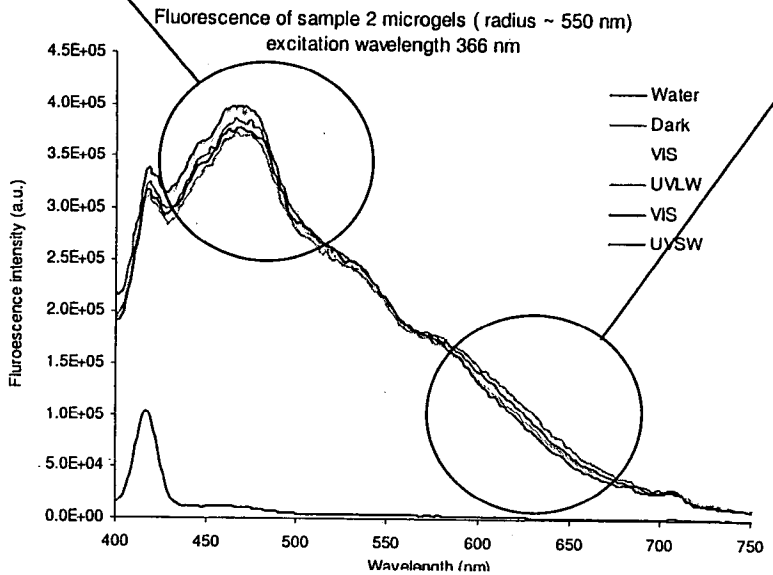
Fluorescence Spectra of sample 2 photoresponsive microgels CR=550nm at 23°C
Sample had been diluted by adding 60 µl sample to 3 ml of water.
Spectrometer settings 366 : 400>800 2 nm /2nm 2nm /0.2 sec



Light scatter may confirm Zhibing's finding that the microgels shrink under UV irradiation. I still need to try to calibrate the scatter in terms of particle size.



Fluorescence spectra show that the spiropyran is undergoing photo-isomerization in the microgels as expected.



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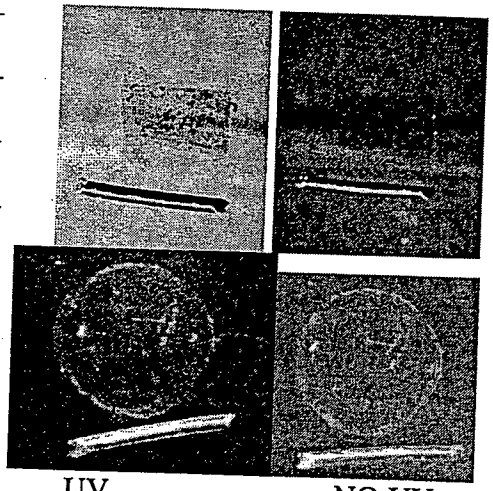
Results from Prof. Zhiliang Hu

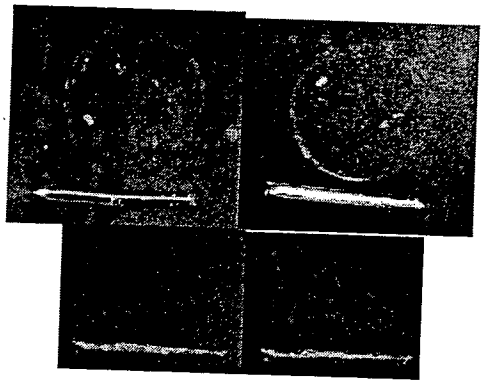
The macrogels expanded on average of ~10% upon UV irradiation. This is a large change for a 1% spiropyran concentration. Also, UV irradiation at 36°C caused the clandiers in the gel to go away. Both these results confirm our bulk measurements shown on pages 10-16

32°C $\frac{d_{UV}}{d} = 1.10$



UV, NO UV

33°C $\frac{d_{UV}}{d} = 1.06$



UV, No UV

Anomalous behavior of spiropyran-gels

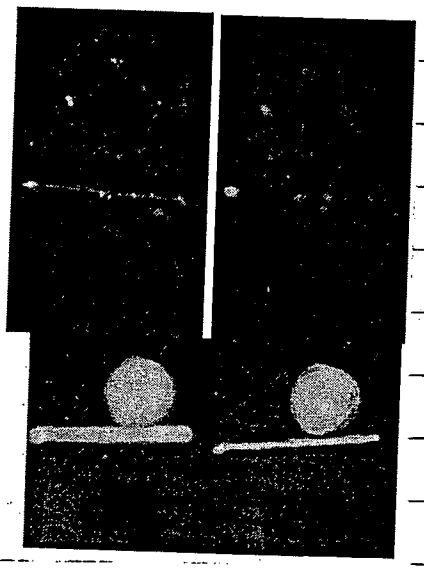
These findings confirm that:

① Macrogels (polymerized at room temperature) expand under U.V. irradiation.

② Microgels (polymerized at 70°C, under visible light) expand under U.V.

③ Microgels (polymerized at 70°C, under UV as in the dark) shrink under U.V irradiation.

36°C $\frac{d_{UV}}{d} = 1.10$



Therefore, it is likely that the polymerization condition affect the spiropyran/polymer and cause it to be organized in different ways.

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Electrophoresis Theory

Electrophoretic mobility $u = v / E$ $\left(m^2 / s\,V\right)$

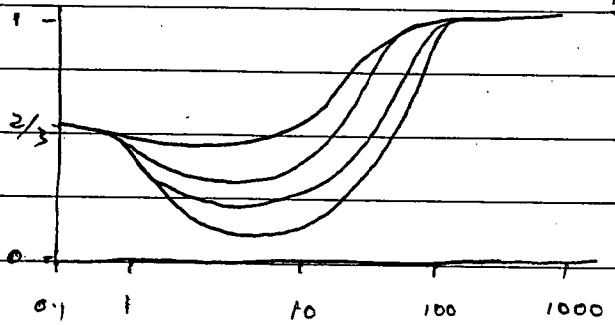
Zeta potential $\zeta = \phi(R') = \dfrac{(q'/R')}{4\pi \varepsilon_r \varepsilon_0} \dfrac{1}{(1+\kappa R')^{***}}$ (V)

Relationship between electrophoretic mobility and zeta potential:

$$\zeta = \frac{c\,\eta\,u}{\varepsilon_r\,\varepsilon_0} \quad (V)$$

Hückel eqn.:

$c = 2/3$ at $\kappa R \ll 1$. Large double layer compared to particle radius



Helmholtz - Smoluchowski

$c = 1$ at $\kappa R \gg 1$. Double layer small compared to particle radius

Electroosmosis Theory

$$\frac{dV}{dt} = v\,A = u\,E\,A = \frac{\varepsilon_r \varepsilon_0 \zeta\, \pi R^2 \phi_{E0}}{\eta\, L}$$

Constants used

$\eta = 0.01$ poise $= 0.001$ Pa S

$\varepsilon_r = 78.5$

$\varepsilon_0 = 8.854 \times 10^{-12}\ C^2 N^{-1} m^{-2}$ or $C^2 J^{-1} m^{-1}$

Volt = Joule / Coulomb = N m / coulomb

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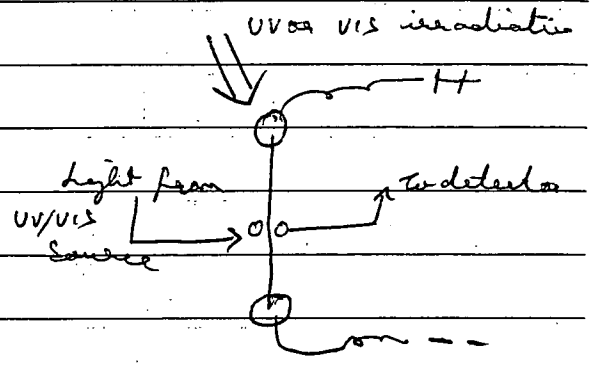
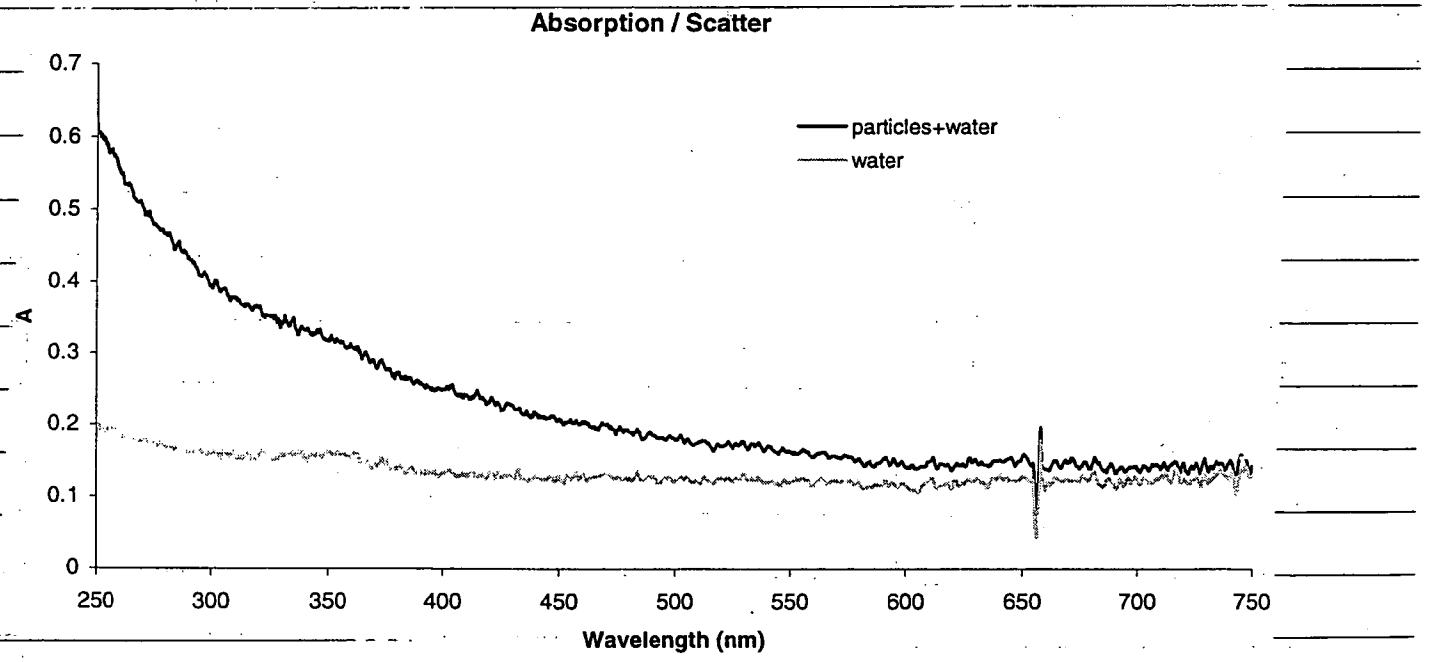
Witnessed	1/01/04	Rohit	11/10/03

Aim: To set up a fiber optic detection system for capelary electrophoresis

Method: A fiberoptic system to detect the movement of particles in a microcapillary was set up as shown in the figure



The capillaries used were 150 mm total length and 75 mm distance to the point of detection. Capillary was of 178 μm ID and 337 μm O.D. Detection was carried out by measuring the absorption/scatter at 200 nm compared to 700 nm



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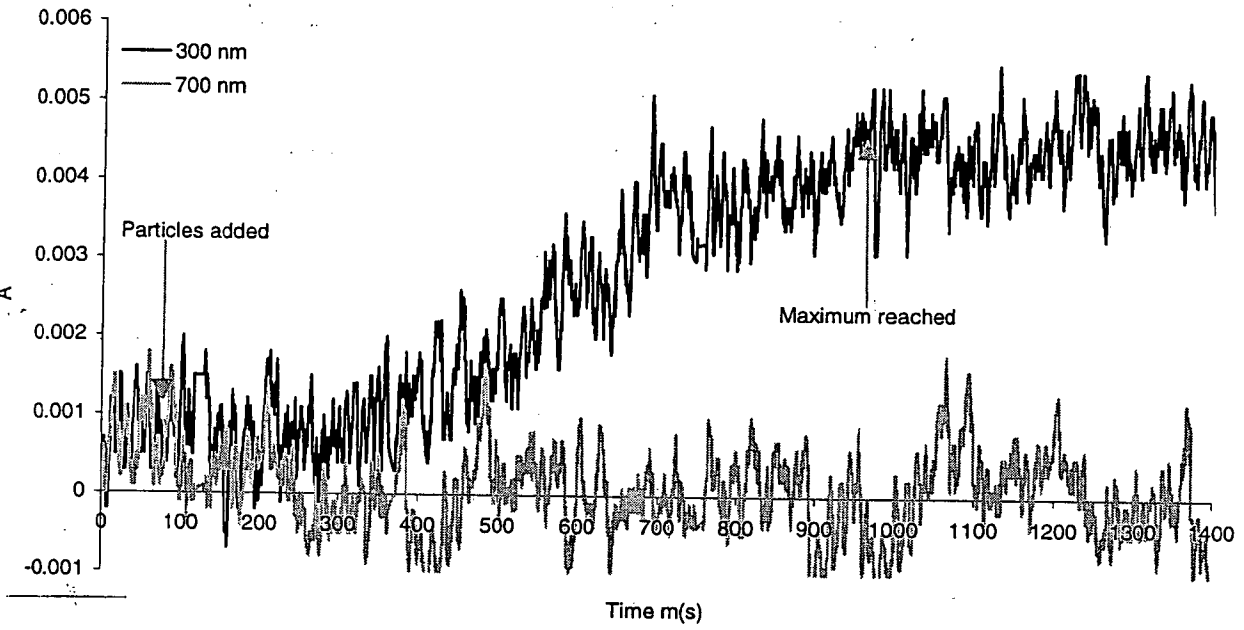
Aim: To measure velocities of UV and VIS irradiated particles in the electrophoretic set-up.

Method: In order to be in the Helmholtz-Smoluchowski regime 30 mM NaCl was used as a buffer. This led to $1/\kappa = 1.76$ nm and $KR = 313$. Potential of 750 V was applied across 150 mm. Particles were irradiated with UV or VIS for 10 min before measurement.

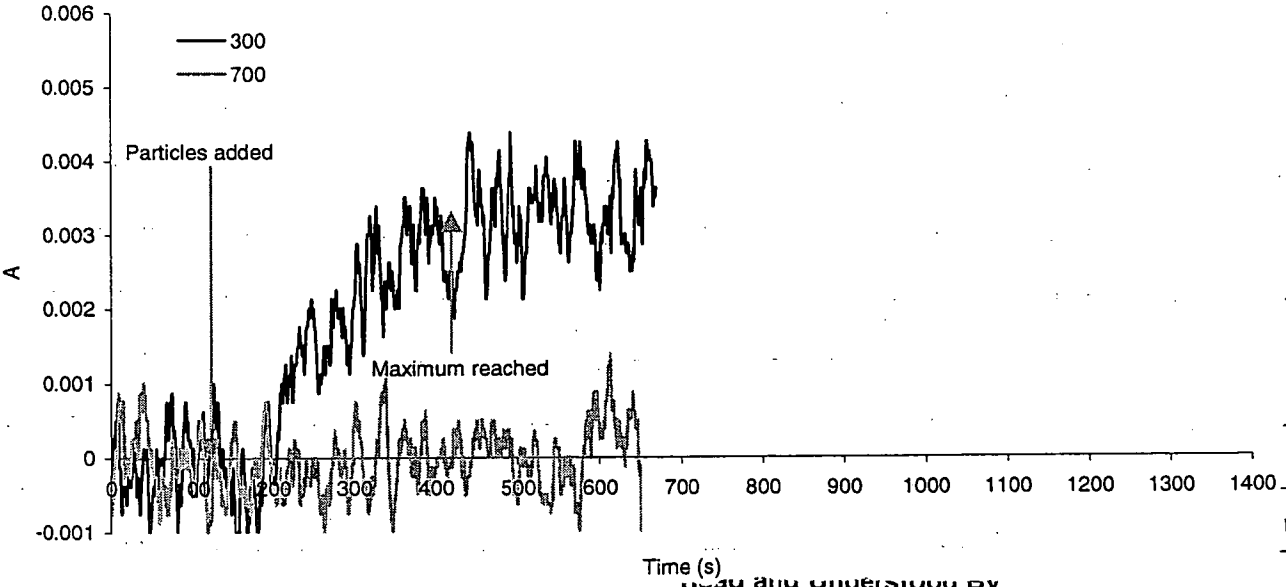
Particles under VIS irradiation



980s − 80s = 890

$v = 84.3 \times 10^{-6}$

Particles under UV irradiation



420s − 120 = 300

$v = 250 \times 10^{-6}$

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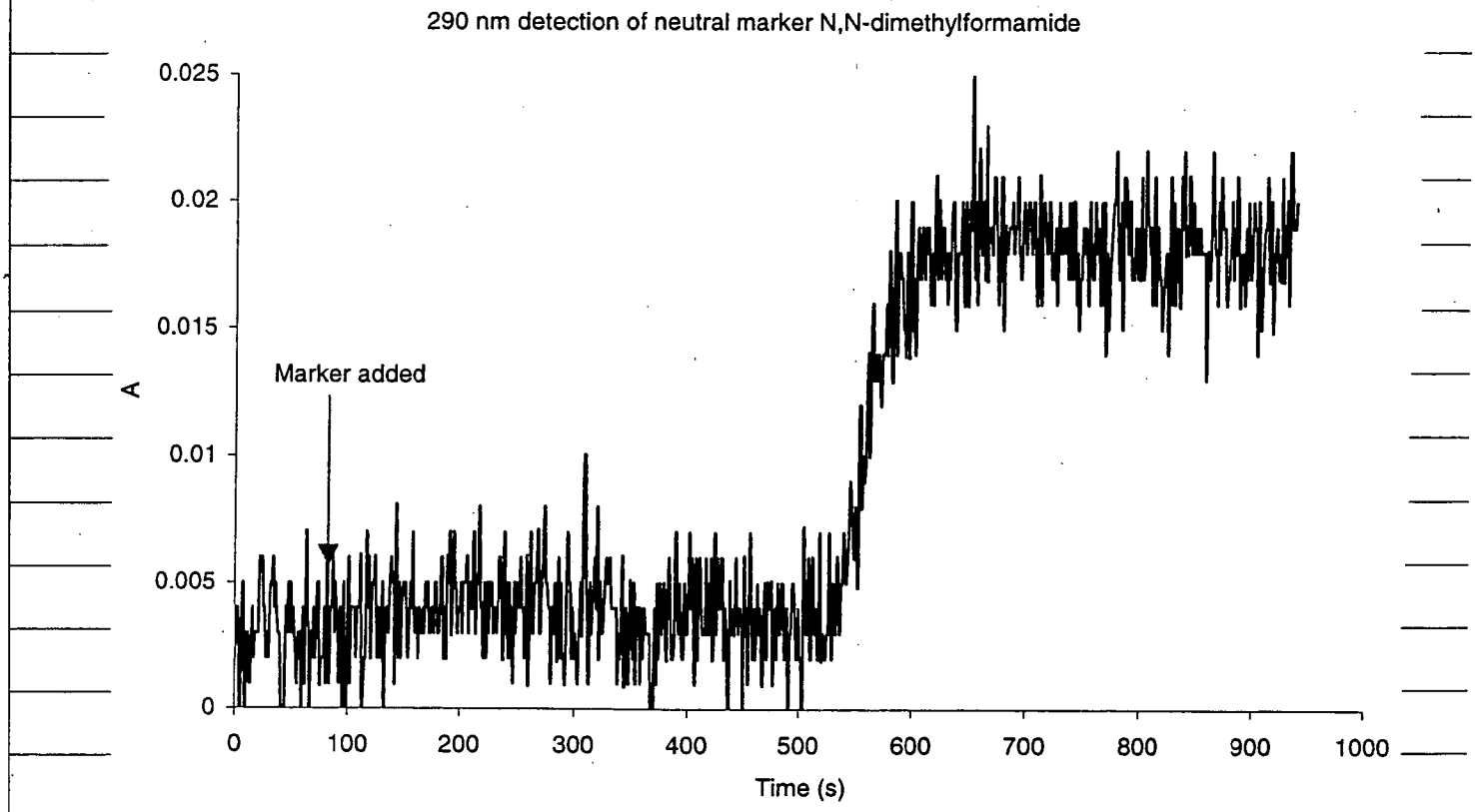
Aim - To measure the electroosmotic flow rate in the electrophoresis set-up using N,N-dimethylformamide as a neutral marker.

Method - The set-up was the same as described on pg 22. Potential of 1600V was applied across 150 mm tube. Absorption at 290 nm was used to detect the presence of DMF. (M.W. 73.09) in 30mM NaCl buffer.

Results -



The DMF took 590 - 120 = 470 sec to reach the window (75 mm length) leading to an electroosmotic velocity of 159.6×10^{-6} m/s. This leads to a calculated value of zeta potential of 22.3 mV. The equivalent zeta potential due to a 750 V potential difference would be 74.9×10^{-6} m/s.

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Electrophoresis calculations:

	VIS	UV
Total velocity v_T	84.3×10^{-6} m/s	250×10^{-6} m/s
Electroosmotic velocity v_{EO}	74.9×10^{-6} m/s	74.9×10^{-6} m/s
Electrophoretic velocity v_{EP}	9.4×10^{-6} m/s	175.1×10^{-6} m/s
Zeta potential	2.7 mV	50.4 mV
Since $1/\kappa \ll R$ we can assume $R \sim R'$ and $q \sim q'$, then		
Approximate surface charge	0.001 C/m^2	0.020 C/m^2
For comparison, on a solid surface (SI conv = 0.88 spheres/nm^2, 10% open)		
Solid surface charge via AFM	0.014 C/m^2	0.028 C/m^2

Assuming that 10% of the spectrozyme open, and no rearrangement of the polymer chain occurs, then the surface concentration of Spectrozyme may be estimated at 1.17 spheres/nm^2 (0.85 nm^2/sphere)

Assuming that 10% of the spectrozyme open, and only open spectrozyme aggregate on the particle surface via polymer chain movement, then the surface concentration of spectrozyme may be estimated to be 0.117 spectrozyme/nm^2 (8.5 nm^2/sphere).

These charge & concentration estimates are based on a hard, homogeneous sphere model. However in our system it is very likely that internal charges can impart the zeta potential either by direct interaction with the electric field, or by coupling to the surface charge by capacitive image charge effects.

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Points to consider regarding use of hydrogels in drug delivery

1. Biodegradability: NIPAAm-based polymers are toxic polymers that are non-biodegradable. They do not form biocompatible or pharmacologically inactive products. An obvious limitation of the normal PNIPAAm hydrogel is its poor mechanical property in a highly swollen state when used as a drug delivery device. Because of its non-biodegradable nature, surgical removal after drug release is desirable.
2. Water content: Hydrogels may absorb upto thousands of times their dry weight in water.
3. Pore size: Labeled molecular probes of a range of molecular weights (MWs) or molecular sizes are used to probe pore sizes in hydrogels. Fluorescein-labeled dextrans are usually used.
4. Volume change: Some hydrogels can reversibly swell or shrink up to 1000 times in volume in response to thermal, pH, and electrically driven stimulii.
5. Charged particles: It has been demonstrated that particles with a diameter up to 10 μm are able to penetrate into the annexes of the skin, i.e. sweat and sebaceous glands and hair follicles (Rolland et al., 1993). The accumulation of triptorelin loaded nanoparticles could create a triptorelin reservoir into the skin. From this reservoir the drug could slowly be released to reach the systemic circulation, generating appropriate plasmatic levels for long time periods. Charged particles are fine for dermal application, however, positively charged surfaces exposed to blood may cause adverse reactions with platlets. Cationic polymers form complexes with anionic DNA and can be used as non-viral vectors for gene therapy.
6. Advantage of responsive nanoparticles: Very quick response to stimulii as compared to polymer membranes.
7. What can be encapsulated : Drugs – Vitamin B12, heparin on the usrface of blood contacting devices, insulin, interferon, anti-glaucoma epinephrine
   Dyes – Methylene blue,
   Enzymes – Immobilized asparaginase
   Antibodies – rabbit IgG
   DNA - reversible cationic gels permit endocytosis followed by intracellular release

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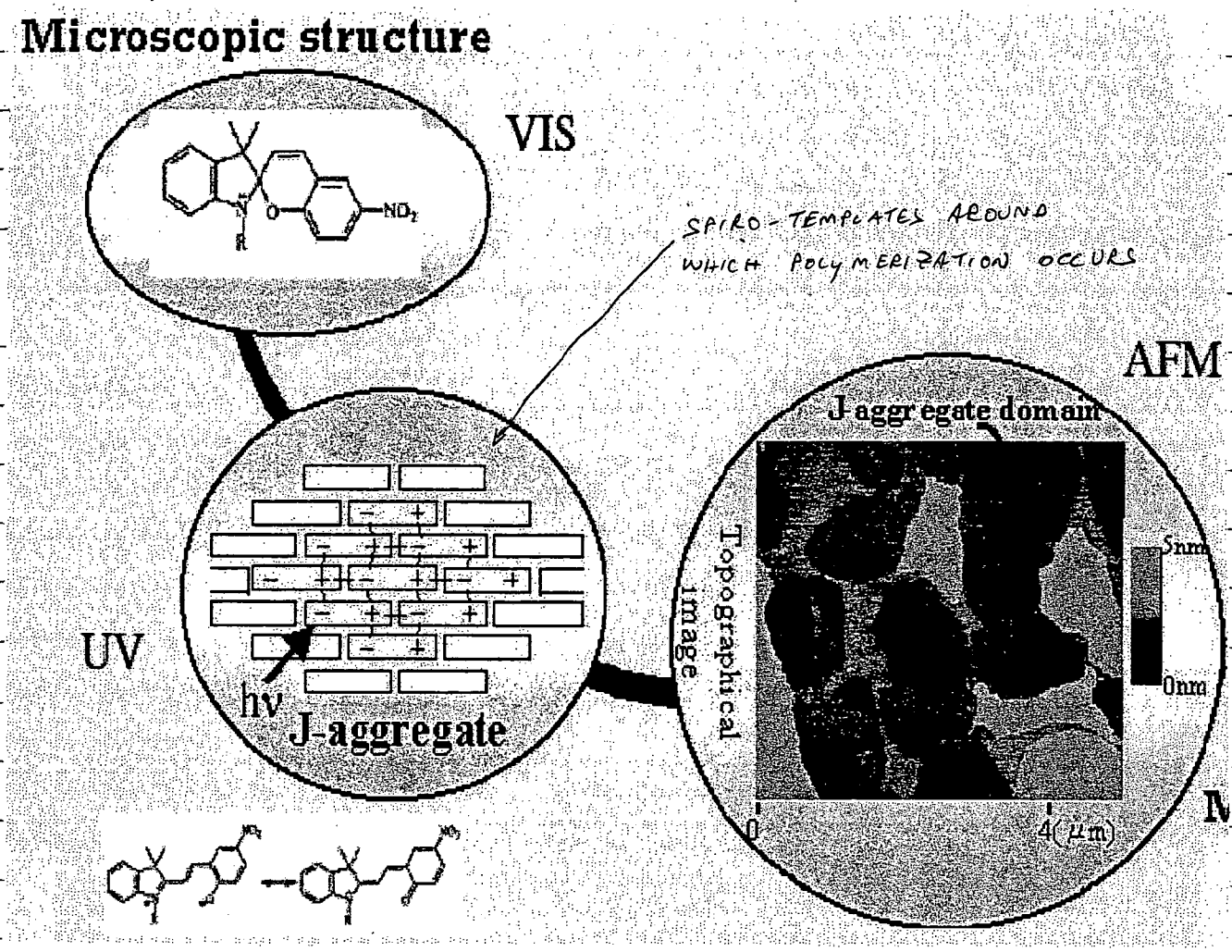
Explanation for why some hydrogels swell under UV & why some shrink



Our current hypothesis for the anomalous behavior of the gels upon UV irradiation is that:

① Gels formed at 70°, in the dark or under U.V. have some of the spiropyran arranged into ordered aggregates such as J-aggregates.

② These gels expand under VIS when the spiropyran closes. Under UV the spiropyran open reforming the aggregate & shrinking

③ Gels that are not formed at 70° & under UV or dark, do not have J-aggregates and hence swell under U.V. due to the increased polarity attracting water molecules.

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PHOTORESPONSIVE METHOD AND APPARATUS FOR DRUG DELIVERY

We claim:

1. A method for the transdermal delivery of a compound, comprising:

using a nanogel as a transport vehicle for a compound, wherein the compound is associated with the nanogel during exposure to ultraviolet light;

exposing the nanogel associated with the compound to the dermis of an animal while the nanogel is exposed to ultraviolet light, wherein the nanogel penetrates a dermal layer; and

removing the ultraviolet light, wherein the exposure of the nanogel to visible light dissociates the compound from the nanogel, wherein the compound is release in a subdermal layer.